TITLE: DUAL-SPECIES BIOFILM BETWEEN *STAPHYLOCOCCUS LUGDUNENSIS* AND *CUTIBACTERIUM ACNES*

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ABSTRACT:

The skin microbiota is composed of several commensal bacteria, including *Staphylococcus lugdunensis* and *Cutibacterium acnes*, which are also associated with opportunistic infections. In the last decade, several studies have been increasingly associating *S. lugdunensis* and *C. acnes* with medical devices-associated infections. This is due to a number of virulence factors, including biofilm formation. Bacterial biofilm contributes to complications on these infections, which often require surgical intervention and aggressive antimicrobial treatment. Recent studies co-isolated *C. acnes* with *Staphylococcus aureus* and coagulase-negative *Staphylococcus*, including *S. lugdunensis*, from these infections. Knowing that the interaction between *C. acnes* and other microorganisms within biofilms is currently largely undocumented and probably underestimated, the aim of this study was to analyze the phenotype produced by these bacteria when they are together in dual-species biofilms with *S. lugdunensis*. For this, biofilm formation was analyzed for *S. lugdunensis* and *C. acnes* separately and combined under anaerobic conditions. We observed that mixed cultures of *C. acnes* and *S. lugdunensis* had a synergic effect on biofilm formation. Growth of *C. acnes* with different *S. lugdunensis* clinical isolates also promoted a stronger biofilm formation, indicating that the phenotype was not isolate-specific. *S. lugdunensis* were grown, and the bacterial supernatant was collected, filtered and concentrated. The impact of this cell-free conditioned medium (CFCM) on biofilm production of *C. acnes* was analyzed. Biofilm production of *C. acnes* grown in the presence of *S. lugdunensis* CFCM showed increased biofilm formation, suggesting that the phenotype was due to *S. lugdunensis* secreted molecules. We then analyzed *C. acnes* and *S. lugdunensis* biofilm formation in titanium surfaces by matrix staining and scanning electron microscope. We observed that mixed cultures formed thicker biofilm in titanium discs than on single cultures and we observed an increased number of *C. acnes* cells in dual species biofilm compared to single-species biofilm. In conclusion, co-culture of these microorganisms under the tested conditions increases total biofilm formation, especially for *C. acnes*, by mechanisms still to be determined. Studying the interactions of these microorganisms in mixed biofilms might be beneficial to understand what is happening during co-infections in prosthetic devices.

Keywords: biofilm, *Cutibacterium acnes*, *Staphylococcus lugdunensis*, prosthetics devices, dual-species biofilm, mixed biofilm.

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